Ranking plant and animal allergens in primary sensitisation based on IgE-binding frequencies, intensities, and clinical relevance in an adult Dutch cohort

To: EAACI VP Science (Professor Óscar Palomares) and EAACI HQ

Type of fellowship: EAACI Research Short-Term Fellowship
Home institution: Research Group for Food Allergy, National Food Institute, Technical University of Denmark, Denmark
Host supervisor: Assistant Professor Dr. Kitty C.M. Verhoeckx
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What questions were addressed and why?
The two main questions addressed were: 1) Can food allergens be ranked based on IgE-binding frequencies or intensities for primary sensitisation? and 2) Does IgE-binding to food allergens correlate to clinical relevance? Currently, there is a lack of data in developing a ranking approach to express the allergenicity of proteins in novel food products relative to that of existing known allergenic proteins. Furthermore, there is currently no reference set of proteins for this purpose, which may hinder the selection of foods to be used for further development and validation of methodologies for novel food proteins capable of causing new food allergies (*de novo* sensitisation) in individuals.

What was the nature of the research?
In this research, we included an adult cohort of 307 patients between 18 to 78 years old who came to the outpatient clinic of the Department of Allergology and Dermatology at the University Medical Centre Utrecht (UMCU, the Netherlands) between January 2022 and September 2023. All patients reported complaints to at least one food item within two hours of consumption. Blood serum from these patients with possible food allergy (with consent) were analysed using the Allergy XPlorer ($ALEX^2$) food panel and processed on the Macro Array Diagnostic X (MADx) 45k Automatic Processing system. We focused on results stemming from 93 unique food items with 157 allergens (76 extracts and 81 components).

What was the result?

The focus of the analysis was mainly on the IgE-binding frequencies, intensities (range 0.1 to 50.0 kU_A/L), and clinical relevance from this cohort. The workflow is outlined in Figure 1.

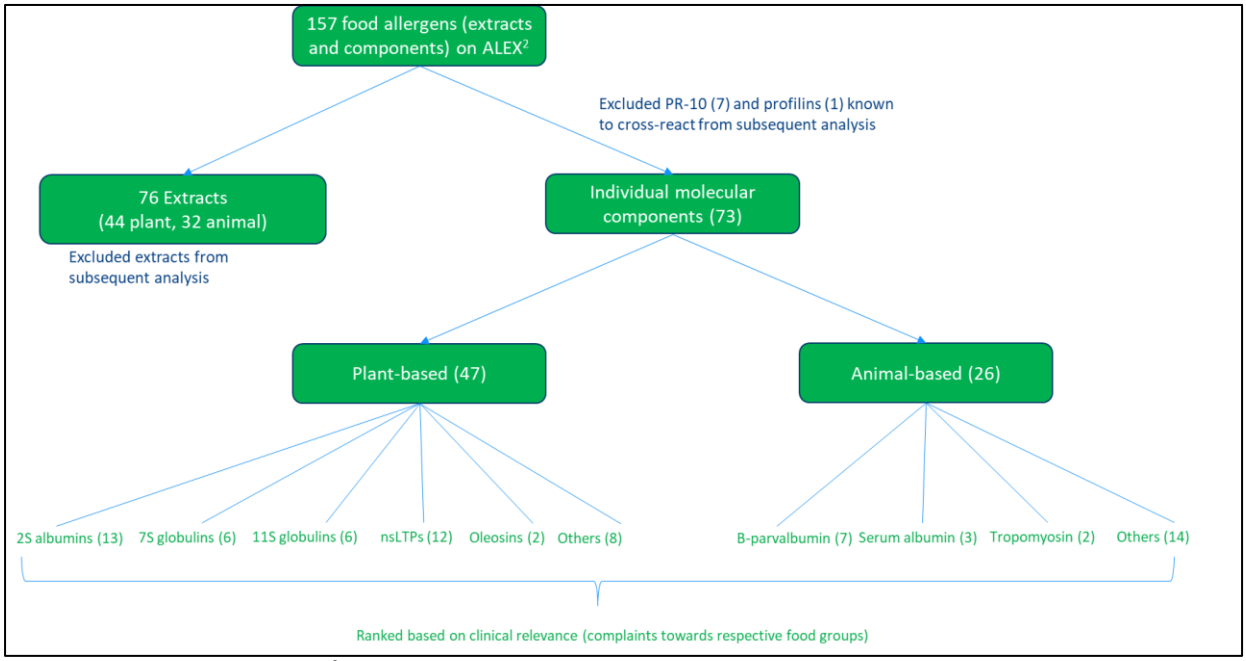

Figure 1: Workflow using the ALEX² panel in the ranking of food allergens based on positive IgE-binding frequencies, intensities, and clinical relevance.

These were the findings of our patients with possible food allergy using the ALEX² panel:

- A ranking was seen when including all allergens (Figure 2)
  - Plant-based PR-10 components showed the highest positive IgE-binding frequencies: hazelnut rCor a 1.0401 (55.4%), apple rMal d 1 (49.5%), strawberry allergen mixture rFra a 1+3 (45.0%), soy rGly m 4 (42.3%), peanut rAra h 8 (33.2%), celery rApi g 1 (25.1%), and carrot rDau c 1 (22.8%). Muskmelon profilin rCuc m 2 followed with a frequency of 12.7%.
  - Since our aim was to rank allergens based on primary sensitisation, and considering the known cross-reactivity with Bet v 1 and Bet v 2 homologues respectively, we excluded PR-10s and profilin components from subsequent analysis.

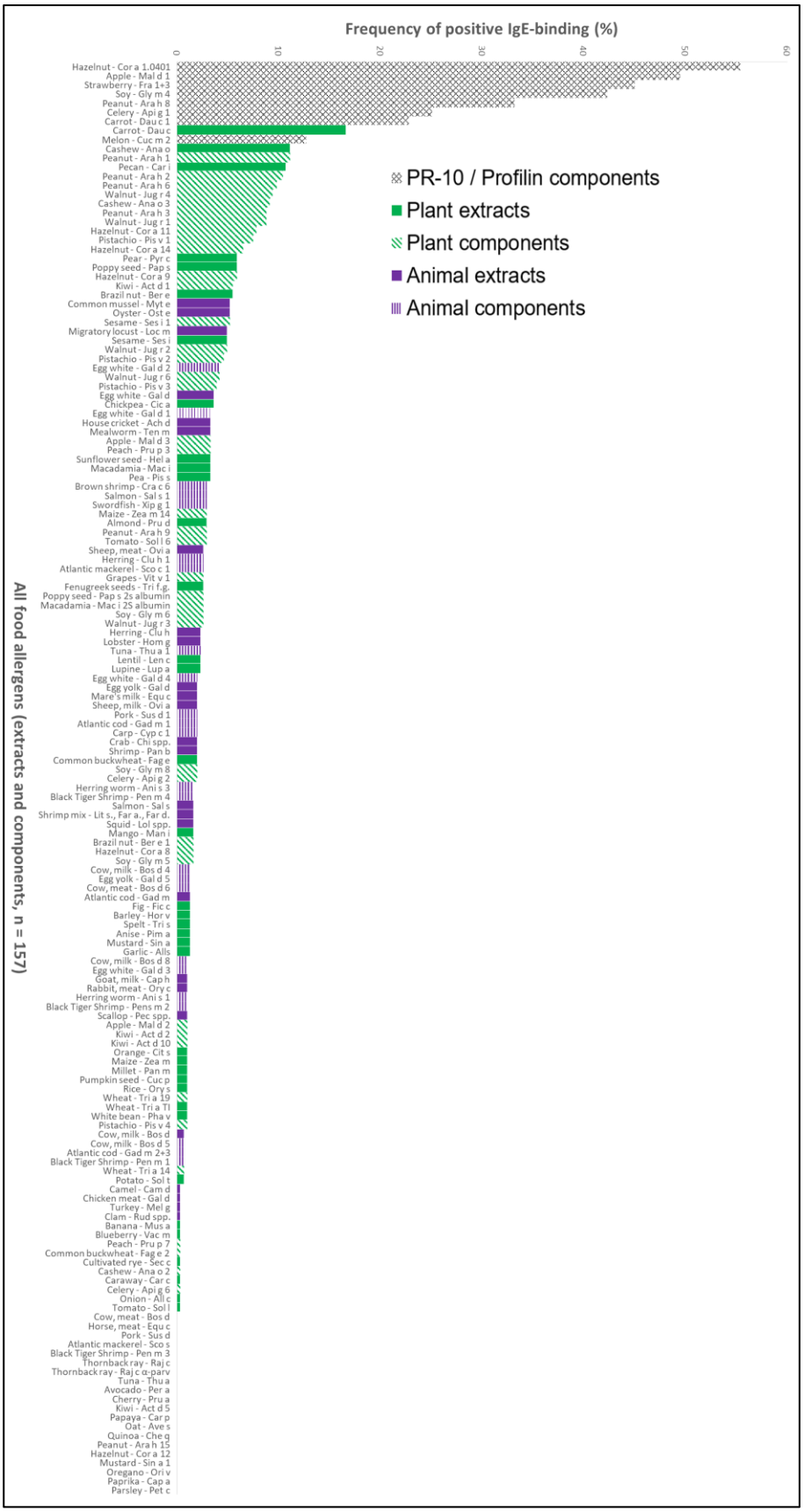

Figure 2. Ranking of 157 food allergens from 93 unique foods based on frequency (in percentage) of positive IgE-binding to sera of Dutch patients (0.3 to 50.0 kU_A/L) with suspected food allergy. Black cross bars indicate PR-10 or profilin allergens, whole green and purple bars indicate plant and animal allergenic extracts respectively, green diagonal lines and vertical purple lines indicate plant and animal allergenic components respectively.

- Ranking of extracts is hampered by incomplete allergen representation and unknown composition
    - Plant-based extracts demonstrated the highest IgE-binding frequencies: carrot (16.6%), cashew (11.1%), pecan (10.7%), pear (5.9%), poppy seed (5.9%), and Brazil nut (5.5%). Among animal extracts, the highest frequencies were observed for common mussel (5.2%), oyster (5.2%), and migratory locust (4.9%).
    - It should be noted that the extract panel was limited and did not include some major allergens such as peanut and hazelnut. Additionally, the exact composition of the extracts was unknown. Therefore, they were excluded from subsequent analysis.

- Ranking of plant-derived components showed 2S albumins with the highest overall IgE frequencies and intensities
    - There were 81 components analysed, including 55 plant-derived (eight of which are PR-10 or profilin-related) and 26 animal-derived.
    - Within the plant-derived groups, 2S albumins showed the highest median IgE intensity levels (kU_A/L) at 4.2, followed by 7S globulins (2.2), 11S globulins (1.4), nsLTPs (1.3), and the "Others" category (1.1).
    - The components with the highest frequencies of positive IgE-binding were rAra h 2 (10.4%), nAra h 1 (11.1%), walnut nJug r 4 (9.4%), rMal d 3 (3.3%), and kiwi nAct d 1 (5.5%) respectively. The components with the highest median IgE intensities (kU_A/L) were rAra h 6 (7.1), rJug r 6 (4.0), nAra h 3 (2.3), rCor a 8 (1.9), and peach rPru p 7 (4.2) respectively.

- Ranking of animal-derived components is hampered by limited allergen representation and low positive frequencies
    - Among animal-derived components, the highest positive IgE-binding frequencies were observed for egg ovalbumin nGal d 2 (4.2%), ovomucoid nGal d 1 (3.3%), and β-parvalbumins from salmon rSal s 1 and swordfish rXip g 1 (2.9%).
    - The components with the highest median IgE intensities (kU_A/L) were black tiger shrimp tropomyosin rPen m 1 (19.0), cow's milk casein nBos d 8 (7.8), and Atlantic herring rClu h 1 (6.8).

- Ranking by clinical relevance showed peanut and walnut with the most symptomatic positive frequencies
    - Food items from the $ALEX^2$ panel were ranked by positive IgE-binding frequencies amongst patients who reported symptoms.
    - Peanut (11.7%) was ranked the highest with symptomatic positive frequencies, with the following component frequencies: nAra h 1 (10.1%), rAra h 2 (9.8%), rAra h 6 (9.1%), nAra h 3 (7.8%), rAra h 9 (1.6%), and rAra h 15 (0%).
    - This was followed by walnut (6.2%), with the components nJug r 4 (3.9%), rJug r 1 (3.9%), rJug r 2 (3.3%), rJug r 6 (2.0%), and rJug r 3 (1.6%).

How will the findings impact future research?

This study advances the understanding of ranking allergenic proteins and extracts from various food groups. It builds on previous work on ranking performed by Smits *et al.*, 2021 on legumes, and Mills *et al.*, 2024 who integrated bioinformatics and literature reviews to rank allergenic proteins of different foods based on clinical relevance. Further research should focus on ranking allergenic proteins and extracts from other food groups to be able to rank known allergenic proteins based on their allergenic potency in causing primary sensitisation. This will allow the development and validation of new methodologies for identifying novel food proteins capable of *de novo* sensitisation. Additionally, these findings will help clinicians in identifying allergenic proteins for primary sensitisation in food allergy-prone individuals. The ALLPreT consortium is also working on purifying allergens from legume superfamilies, and this work will provide directions for specific allergens in legumes to focus on.

These findings will be presented at the 4th Food Allergy Forum in Amsterdam, the Netherlands from 22 to 24 September 2025, and are intended for submission to EAACI's Allergy journal.

Personal reflections

I received a lot of insights from people in the department who have extensive expertise both in the research and clinical settings. I always wanted to work in a hospital to help contribute to the people affected by food allergy and making their quality of life better. The main thing I learnt was that while we made a lot of figures to explore the many different types of data we could show to the audience, it would have been better to focus on the main message we wanted to get across right from the start, and the relevant figures (which we eventually did in the last month). Different organisations use different statistical programmes, so I was lucky enough to add learning how to operate SPSS and create syntaxes to my repertoire of statistical software! In addition, when I found out that I received the EAACI Research Fellowship 2025, I was extremely surprised and happy, and it served as a very good motivation for me to put in even more effort and enthusiasm in our project. To me, food is an international language that brings everyone together, regardless of who they are. I would like to contribute to this cause by making food safe so that people from all walks of life can enjoy the wonderful pleasures of eating.

Acknowledgements

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For future Junior Member applicants, please take the time to apply for the Junior Membership and EAACI's Research or Clinical Fellowships. I was fortunate enough to have been considered for multiple networking and research opportunities by EAACI. The organisation is here to help contribute towards the better quality of life, be it in food allergy or immunology!